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Plasma Catecholamine and Corticosterone Responses to Predictable and Unpredictable Noise Stress in Rats[1]

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DE BOER, S. F., J. VAN DER GUGTEN AND J. L. SLANGEN. *Plasma catecholamine and corticosterone responses to predictable and unpredictable noise stress in rats.* PHYSIOL BEHAV **45**(4) 789–795, 1989.—Plasma noradrenaline (NA), adrenaline (A) and corticosterone (CS) increases were determined in individual rats subjected to either 20 regularly or irregularly scheduled white-noise stimulations (4 min, 100 dBA). Blood was frequently sampled during the first and twentieth noise exposure, and during a reexposure after 24 hr. During the sampling periods, behavioral activities of the rats were recorded. The initial noise-induced CS release was partially reduced following the regular noise presentations. The increase after irregular presentations remained high. The difference in adrenocortical responsiveness between regular and irregular exposure persisted for 24 hr. The NA response to noise was partially attenuated following irregular administration of noise. However, regular exposure produced increased NA levels prior to noise presentation and a subsequent decrease during stimulation. After 24 hr, noise evoked an exaggerated initial NA release in the regular group. The noise-elicited rise in A was completely abolished after 20 noise presentations irrespective of whether these were applied regularly or irregularly. Reexposure after 24 hr evoked again a significant A response in both groups. No differences were observed in the habituation pattern of behavioral reactions among the regular and irregular groups. The results show that the sympathetic neural, adrenomedullary and adrenocortical systems differ in degree and speed of adaptation to intermittent stressful stimuli and in sensitivity to the predictability of stressors.

Noradrenaline Adrenaline Corticosterone Predictability White-noise Adaptation Stress Rat

ACUTE arousing or stressful stimuli are associated with increased activity of both the pituitary adrenocortical system and the sympathetic adrenomedullary systems, resulting in raised plasma concentrations of the glucocorticoid corticosterone (CS) and of the catecholamines noradrenaline (NA) and adrenaline (A) (4,14). Repeated presentation of the same type of stressor over time can lead to a gradual decrease (3, 10, 17, 26, 27, 31), to no change (7, 33, 41) or to an increase (6, 8, 11, 26, 41) in the responsiveness of these neuroendocrine systems. Due to a wide variation in organismic and procedural parameters across studies, the factors responsible for producing the different patterns are not readily apparent. But the type, severity and frequency of the stressor are generally considered to be major determinants of the direction, degree and/or rate of the response change. Recent parametric work supports this supposition (24, 25, 31, 34, 35).

Besides the physical properties of the stressor, the ability of the organism to predict some characteristic (onset, termination, duration) of the stressful event may be an important psychological variable influencing the quantitive and qualititive aspects of the neuroendocrine response pattern to repeated stress. Predictability can be operationalized effectively in paradigms employing repeated administration of identical stimuli by manipulating the variability of the interval between successive stimulations (time discrimination) (20, 21, 23).

Using such an approach on a long-term basis (across days), it was shown that the CS levels following restraint (36) or novelty stress (28,29) were of greater magnitude in rats which were previously exposed to irregularly scheduled sessions of restraint or novelty as compared to animals which had experienced the same stress in a regular fashion. Further, it was found that the effects of prenatal stress on subsequent motor development and behavior of the offspring were dependent on the schedule (regular or irregular) of stressor administration during pregnancy (18). In a short-term procedure (within 24 hr), it was demonstrated that rats receiving a series of brief electric footshocks on a regular (fixed-time) schedule had lower CS levels (7) and developed less gastric pathology (20) than another group that received identical shocks on an irregular (variable-time) basis. These results emphasize the (patho)physiological importance of seemingly subtle schedule parameters on the process of adaptation to repetitive stressful stimuli.

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The present study was designed to evaluate more specifically the role of this predictability factor upon the adaptation of both sympathetic adrenomedullary and pituitary adrenocortical reactivity to short-term repeated stressor presentations. Therefore, the changes of noise stress-induced plasma NA, A and CS responses were determined in individual rats exposed to a series of regularly (predictable) or irregularly (unpredictable) scheduled "white" noise stimulations. The data reported indicate that stressor predictability differentially influences the CS and NA response patterns to repeated stressor presentation, and does not affect the A response.

METHOD

Animals and Housing

Male Wistar rats (CPB-TNO, Zeist, The Netherlands) weighing 265–290 g on their arrival in the laboratory were housed individually in clear Plexiglas cages (25 × 25 × 30 cm) on woodshavings. Subjects were in full view, sound and smell of each other and had free access to food and water at all times. Cages were placed in a room under conditions of constant ambient temperature (21 ± 2°C) and a fixed 12-hour light/12-hour dark photoperiod (lights on at 0700) for two weeks prior to surgery. The rats were accustomed to the presence of the experimenter by daily handling for ± 30 sec.

Surgery

Surgery was performed under complete ether anesthesia. All experimental animals were equipped with a silicon cannula (i.d. 0.5 mm; o.d. 1.0 mm) into the entrance of the right atrium (venae cava) via an external jugular venotomy and externalized on the top of the skull according to the techniques originally described by Steffens (38). This method allows frequent withdrawal of small amounts of blood without disturbing the animals either behaviorally or physiologically (38,42). After surgery, the rats were allowed to recover for at least one week before the start of the experiments. During this period animals were weighed every morning at 0900 and were connected to the blood sampling tubing several times in order to accustom them to the blood sampling procedure.

Apparatus

White noise (100 dBA; 0.05–26 kHz) was generated by a Grason-Stadler white noise generator (model 901B) and delivered to a 15-W loudspeaker (∅ = 20 cm) located 50 cm above the animal's cage. A precision sound level meter (Brüel and Kjaer, 2203) was used to adjust the intensity of noise inside the cage at the approximate position of the rat. Background noise level, produced by the ventilation system inside the room, was 44 ± 3 dBA. The time conditions of the experiment were controlled by programmable timers (Campden Instruments, model 249 and 263) controlling the onset and duration of the white noise tone as well as the interstimulus intervals.

Experimental Procedure

The rats were randomly divided into a regular group (n = 6), an irregular group (n = 6) and a control group (n = 4). A rat from one of these groups was studied separately on two successive experimental days. The conditions were randomized over the whole experiment (16 animals) which took a total of 32 days. Experimentations were performed between 0900 and 1700 hr on the first day and between 0900 and 1030 on the second experimental day. Individual rats of the regular group were subjected to 20 regularly scheduled noise exposures (4 min duration, 100 dBA, fixed interstimulus interval of 16 min). The rats of the irregular group were subjected to 20 irregularly scheduled noise exposures (4 min duration, 100 dBA, variable interstimulus interval with a mean of 16 min, range 2–30 min). Subjects of the control group of rats were only exposed twice to the noise stimulus with an interval of 380 min, i.e., at the times of the day when the other two groups of rats received their first and twentieth noise exposure, respectively. Twenty-four hours after the first noise exposure, animals of all three groups were reexposed once to the same noise stimulus. During the first and twentieth noise exposure on day 1 and during the reexposure on day 2, blood samples of 0.35 ml were taken 4 min before (basal or prestimulation values) and 0.5, 3.5, 8, 12 and 16 min after onset of noise (response values). Immediately after each sample, an equal volume of heparinized blood freshly obtained from a cannulated donor rat was transfused through the catheter. The three noise exposure trials during which blood samples were taken are named in the text as sampling trial 1, 2 and 3, respectively.

During 30 sec before and 30 sec after each blood sampling, the occurrence of the following behavioral elements were rated manually and afterwards classified into four behavioral or motor activity scores: 1) resting: lying down quietly in a stretched or curled up-like position with eyes open or closed; yawning and stretching may occur; 2) alert: sitting, slow movings through the cage, scanning, sniffing; 3) active: rearing, grooming, scratching, eating and drinking; 4) excited: fast bursts of locomotor activity, burying/shoveling in bedding material, freezing.

Chemical Determinations

The blood samples were immediately transferred to chilled (0°C) centrifuge tubes containing 8.5 µl of a heparin solution (500 IU/ml) as anticoagulant. For the determination of plasma catecholamine (CA) contents, an aliquot of 250 µl transferred blood was immediately pipetted into chilled tubes containing 12.5 µl of a solution of 22 mg/ml disodium EDTA and 24 mg/ml reduced glutathione in order to prevent CA degradation. The remaining 100 µl blood was used for the CS assay. After centrifugation (4000 × g for 10 min at 4°C), supernatants were removed and stored at −30°C.

NA and A concentrations were measured in duplicate in 20 µl perchloric acid-deproteinized plasma according to a radioenzymatic COMT-procedure (40). The CAs were converted into their [^{3}H]-methoxy derivatives by incubation with S-adenosyl-L-[methyl-^{3}H]methionine (80 Ci/mmol; NEN Chemicals) in the presence of catechol-O-methyltransferase. Labeled products were isolated by organic extraction and paper chromatography. After elution of the labeled products, activity was counted in a liquid scintillation analyzer (Philips, The Netherlands). CA concentrations were calculated from net DPM values for samples and internal standards and were expressed as pg/ml. The intra and interassay variability were less than 10% and 15%, respectively. The sensitivity of the assay (amounts corresponding to twice the blank) was 2 pg for NA and 1 pg for A.

Plasma CS concentrations were determined in duplicate according to a competitive protein-binding method (32). Corticosterone was extracted with dichloromethane from 25 µl samples of plasma and the dry residue was incubated with a corticosteroid-binding globulin tracer solution [0.1% plasma from adrenalectomized female rats containing [1,2-^{3}H]-corticosterone (40–50 Ci/mmol; NEN Chemicals) as tracer]. Unbound steroid was removed using dextran-coated charcoal. Standard CS was supplied by Sigma. The intra- and interassay coefficients of variation were less than 10%. Fifty percent displacement of tracer steroid was

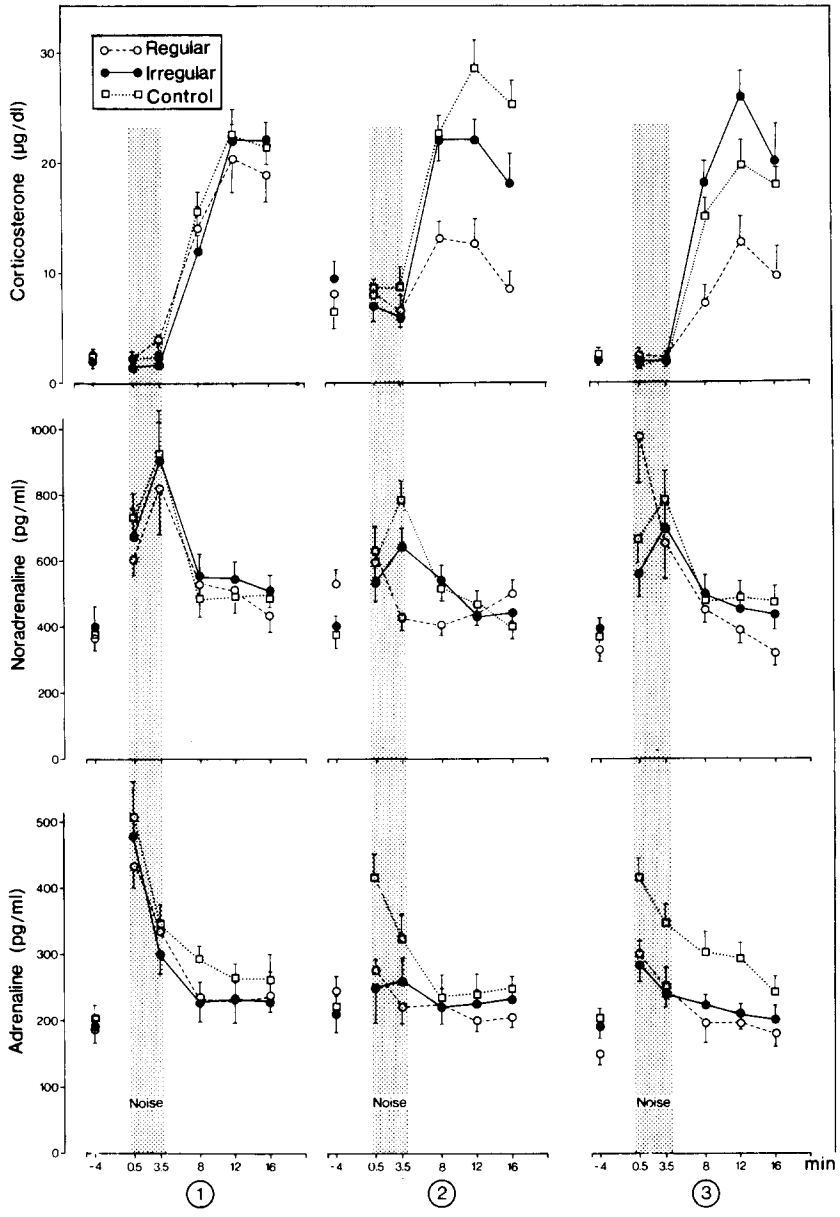

FIG. 1. Mean (±SEM) time course of changes in plasma corticosterone, noradrenaline and adrenaline concentrations in response to 4 min white noise stimulation (100 dB) during sampling trials 1 (first exposure), 2 [twentieth or second (control) exposure] and 3 (reexposure after 24 hr) for the groups of rats in the regular, irregular and control conditions. For results of ANOVA see text.

obtained at a concentration of 22 ± 1 µg/dl.

Statistical Analysis

The response patterns of each hormone as well as the pattern of the behavioral activity scores were first evaluated by a three-way mixed design ANOVA, utilizing one between-subjects factor (group) and two repeated measures within-subjects factors (sampling trial and sampling time). The group factor had three levels (regular, irregular and control) and the two within-factors had three and six levels respectively. The multivariate model was used for the repeated measures where appropriate and possible (12,43). In case of significant interactions, separate ANOVAs were performed for each trial and/or for each group. Further analysis was made by post hoc *t*-tests to determine the source of detected significance in the ANOVAs. The criterion of significance was set at $p<0.05$.

RESULTS

Figure 1 shows the mean time course of changes in plasma corticosterone, noradrenaline and adrenaline concentrations in response to 4 min of white noise stimulation during sampling trials 1 (first exposure), 2 [twentieth or second (control) exposure] and

3 (reexposure after 24 hr) for the regular, irregular and control condition.

Corticosterone

ANOVA revealed significant main effects of trial, $F(2,12) = 5.67$, and time, $F(5,9) = 36.8$, but not groups. There were significant two-way interaction effects of group × trial, $F(4,26) = 3.16$, and trial × time, $F(10,130) = 4.63$, as well as a significant triple interaction group × trial × time, $F(20,130) = 1.85$. A set of three additional ANOVAs, one for each trial, revealed that there was a significant effect of time but not of groups for each analysis. Significant group × time interactions were observed only for trial 2 and 3. Between-group and between-time comparisons showed that the prestimulation resting levels ($t = -4$ min) and the values at $t = 0.5$ and 3.5 min during the noise stimulation period did not differ from each other at any sampling trial. This indicates that the plasma CS contents remained at basal (prestimulation) level during the stimulation period of 4 min. Between-trial comparisons of these same means revealed higher values at sampling trial 2, due to the normal circadian rhythmicity of basal CS release under our laboratory conditions (15). All three groups of rats during all sampling trials showed that their response values at $t = 8$, 12 and 16 min were significantly elevated above the prestimulation resting level, indicating that the CS response to noise stimulation during each trial is delayed. The magnitude of the CS increase was different between groups as well as between sampling trials: The response values of the irregular and control animals did not differ, compared either within or between both groups. In contrast, during the second and the third sampling trial the response values of the animals in the regular group were significantly reduced as compared to the first trial, and were also significantly lower than those for the irregular and control group.

Noradrenaline

ANOVA showed significant main effects of trial, $F(2,12) = 4.71$, and time, $F(5,9) = 8.28$. Neither the main effect of group nor the interaction effect group × trial approached significance. Reliable two-way interactions were observed between group and time, $F(10,20) = 2.87$, and between trial and time, $F(10,130) = 3.9$. The three-way interaction effect was also found to be significant, $F(20,130) = 2.89$. Separate ANOVAs on each trial revealed significant time effects for trial 1 and 3, but not for trial 2. Only trial 2 and 3 showed a group × time interaction. Subsequent between-group and between-trial comparisons indicated that the prestimulation resting level of the regular group during the second sampling trial was higher than the corresponding values for the irregular and control groups and that the values of the first and third sampling trial were different. At no time point during all three sampling trials were there significant differences between the irregular and control group: Upon noise exposure NA increased immediately and peaked at $t = 3.5$ min, whereafter the values declined towards resting levels. In contrast to the control group however, the irregular group displayed a small but significant reduction of the response values at $t = 0.5$ and 3.5 min during both the second and third trial in comparison with the first sampling trial.

During the second trial the temporal response pattern of the regular group differed from the two other groups: Upon noise exposure no significant increase above the already heightened prestimulation value occurred at $t = 0.5$ min and subsequently, instead of a further increase like the irregular and control group, a significant decrease was observed at $t = 3.5$ and 8 min, whereafter at $t = 16$ min the NA values were slightly increased again towards prestimulation level. Upon noise onset during the third trial the regular group of rats showed a markedly enhanced response at $t = 0.5$ min when compared with the other two groups or compared with the other two sampling trials.

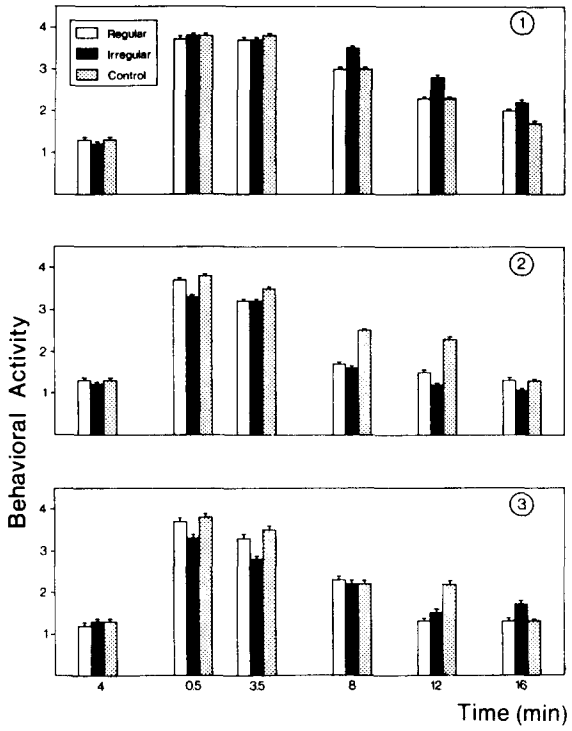

FIG. 2. Mean behavioral activity scores during sampling trial 1, 2 and 3 for animals subjected to the regular, irregular and control condition. Numbers on the ordinate refer to the animal's behavioral repertoire ranging from the quiet state 1) to alertness 2), active 3) and finally to excitement or agitation 4). For further details see text.

Adrenaline

Analysis yielded significant effects of trial, $F(2,12) = 6.36$, time, $F(5,9) = 14.9$, as well as a significant trial × time interaction, $F(10,130) = 9.7$. Neither the group effect nor the interaction effects group × trial and group × time reached statistical significance. A reliable group × trial × time interaction was found, $F(10,130) = 3.56$. Separate ANOVAs were performed for each trial. Only trial 3 showed a main effect of group. Although all analyses showed a main effect of time, the interaction group × time was significant for trial 2 only.

There were no significant differences between the regular and irregular groups of rats at any time point during any of the three sampling trials. During the first sampling trial, plasma A increased immediately after noise onset and peaked at $t = 0.5$ min. Subsequently the A contents declined and returned towards basal level after stimulus offset at $t = 8$–16 min. During the second trial, however, the regular and irregular groups showed no significant A response to noise anymore, in contrast to the control group which responded with a similar A increase as compared to the first trial. During the third trial the regular and irregular group responded significantly to noise, but the A elevation at $t = 0.5$ and 3.5 min was significantly reduced as compared to the first trial as well as when compared with the control group.

Behavior

Figure 2 shows the mean behavioral activity scores over time

during sampling trial 1, 2 and 3 for three experimental groups of rats. In the ANOVA, significant effects of trial, $F(2,12)=19.5$, time, $F(5,9)=85.4$, and of the interaction trial × time, $F(10,130)=3.56$, were obtained. No significant main or interaction effects involving group were found, indicating that there were no significant between group differences for the time points during each of the three trials. Immediately upon noise onset, the rats startled, whereafter the animals became either very active, displaying alternating short-lasting episodes of rearing, burrowing, shoveling bedding material with their snouts and forepaws, and grooming or remained relatively motionless (freezing) in an erect posture for a few minutes, whereafter they cautiously began scanning/rearing and/or grooming. After noise offset rats stayed alert and/or active for about 5–10 min, and subsequently returned to the resting state. During the second and third sampling trial the initial (startle-like) behavioral reaction was similar to the first trial, but a significantly faster decrease in the behavioral activity scores at $t=3.5$–16 min was observed in comparison with the return during the first trial for the regular, $t(5)=2.69$–5.97, $p<0.05$, and irregular group, $t(5)=2.71$–4.00, $p<0.05$. Such a diminished behavioral response was not observed in the control group.

DISCUSSION

The present study confirms our previous findings (14) that acute brief exposure to "white" noise elevates plasma A, NA and CS concentrations, each in its own specific temporal pattern. It is assumed that this stimulus coupled neuroendocrine response profile reflects a component of an unconditioned orientation/arousal or stress reaction of the animals.

Further, the data confirm the earlier finding that repeated exposure to a noise stimulus results in adaptation of the pituitary adrenocortical and sympathetic adrenomedullary responsiveness, this being reflected in a reduced plasma CS, NA and A response. However, the rate of the response decrements differs among the hormonal variables and, in the case of plasma CS and NA, strongly depends on the schedule of stressor administration. With regard to the adrenocortical axis, the partial reduction of the noise-induced CS release was only observed following a regular (fixed time intervals) schedule of noise presentations. In contrast, no such adaptation effect occurred when the same noise stimuli were delivered on an irregular (variable intervals) basis: animals continued to respond to noise with a comparable CS increase as during their initial exposure despite repeated presentations of the identical stimulus over time. Interestingly, the observed difference in pituitary-adrenocortical responsivity between the regular and irregular group persisted for 24 hr when the animals were reexposed to the noise stimulus. These findings are generally in line with previous observations that the pattern of exposure to a stressful stimulus, affects the pituitary-adrenocortical responsiveness, i.e., the highest CS level occurs following irregular (unpredictable) stressor presentations (7, 28, 29, 36).

Concerning the sympathetic adrenomedullary systems, the results show a complete reduction of the plasma A response already following 20 repetitive noise presentations irrespective of whether these were applied regularly or irregularly. A single presentation of the stimulus after 24 hr evoked again a significant A response, the magnitude of it being similar for both groups of rats but smaller as compared to their initial response.

In the case of NA, a different picture emerged: following irregular administration of noise, a partially attenuated but otherwise normal temporal pattern of plasma NA elevation was observed. Following regular presentation however, there was an increased resting level prior to the twentieth exposure and a decrease instead of an increase during stimulation. Repetition of the noise stimulus after 24 hr evoked a suprisingly exaggerated initial NA release in the regular group as compared to the other two groups of animals.

It is clear that the adaptation profiles of NA and A observed in this study differ in several aspects. Although a minor portion of circulating NA may have an adrenomedullary origin, numerous studies have reported that the plasma NA level is a sensitive indicator of the functional activity of the sympathetic nerves whereas plasma A serves as a reliable index of adrenal medullary discharge (19,37). If one accepts this view, it can be concluded that the neural and adrenomedullary branches of the sympathetic nervous system differ in the degree or speed of adaptation to intermittent stressful stimuli and in the sensitivity to alterations in the regularity (predictability) of stimulus presentation. These findings support the notion that the activity of the two parts of the sympatho-adrenal system could be regulated by separate brain mechanisms attuned to the specific characteristics and/or requirements of the imposed conditions.

The relationship between predictability of aversive (or appetitive) events and (patho)physiological functioning has received considerable attention in the literature and the obtained results have been explained by a variety of, partially overlapping, terms and concepts [see (1, 2, 23) for reviews]. In most of the interpretations it is argued that unpredictable (e.g., irregularly applied) aversive events are more stressful and provoke more arousal or anxiety than predictable (e.g., regularly applied) events. This view is consistent with the behavioral result that given a choise, animals prefer fixed-time (regular) shock schedules over otherwise identical variable-time (irregular) shock schedules (5). Indeed, the different NA and CS response patterns observed in the regular and irregular groups might be explained by assuming that animals from the regular group developed an expectancy or anticipation of the stressor, following regular exposure to it, and thus were able to respond more effectively to the event (to reduce its aversive, disturbing or deleterious impact). It is known that animals can use the passage of time as a conditioned/discriminative stimulus (22). Adopting this view, the NA response pattern following repeated regular noise presentation could be interpreted as an anticipatory or expectancy effect of the sympathetic nervous system, i.e., increased activity prior to the twentieth exposure and an enhanced initial reactivity during the reexposure 24 hr later. Due to subsequent reduction in the aversiveness or arousing impact of noise in the predictable (regular) situation, a decrease in the adrenocortical response magnitude (or a faster rate of adaptation) is seen. This interpretation is also compatible with the physiological function of corticosteroids in stress, i.e., that a CS response has the general function to protect the organism from its own rapid (sympathetic adrenomedullary) reflexes to stress-induced disturbances of homeostasis (30).

No differences were observed in the adaptation pattern of behavioral reactions between the regular and irregular groups. Therefore, on a behavioral level no evidence was obtained that rats from the regular group engaged in behaviors or postural adjustments which might have reduced the arousing nature of the noise stimulus other than those observed in the irregular group of animals. However, measurement of behavioral motor activity in more quantitive ways (e.g., intensity of the initial startle reflex), might have revealed differences. Indeed, Davis (13) has shown that startle responsiveness was lower following a series of tone stimuli with regular as compared to variable interstimulus intervals.

There appears to be a striking similarity between the process of neuroendocrine adaptation to stress and the process of behavioral and neurophysiological habituation to a sensory stimulus as to the factors determining the pattern of response change. For these processes the properties of the stimuli (intensity, duration, frequency) as well as the parameters of the interstimulus interval

(length and variability) are of similar importance. For example, responses to intense stimuli habituate at a slower rate than responses to less intense ones, and responses to frequently delivered stimuli habituate faster than responses to the same stimulus presented less often (24, 25, 34, 35, 39). From the behavioral/neurophysiological habituation literature it has also become evident that habituation rate is influenced by the variability of the interstimulus intervals (ISIs): Variable ISIs (implicating stimulus uncertainty or unpredictability) usually delay habituation more than constant ISIs (13,39). Our present finding that the adaptation pattern of CS and NA to repeated stressor presentation is similarly dependent on the latter parameter gives further support to the theoretically attractive hypothesis of Natelson and colleagues (34,35) that "stress adaptation" is (at least partially) similar to the process of "sensory habituation."

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